

ON THE PATHOGENICITY OF A
 TRYPANOSOME (*T. RHODESIENSE*,
 STEPHENS AND FANTHAM) FROM
 A CASE OF SLEEPING SICKNESS
 CONTRACTED IN RHODESIA

BY

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The question as to whether more than one trypanosome acts as a pathogenic agent in man is one which in the past has been much discussed.*

The findings of practically all workers are agreed in deciding that the trypanosomes infecting human beings in different parts of tropical Africa are identical.

Recently, however, doubt has been expressed regarding the identity of a human trypanosome of Rhodesia with that of *T. gambiense*†.

In December, 1909, a patient, W.A. (European), suffering from trypanosomiasis, was admitted into Major Ross's clinic in Liverpool.

So far as could be ascertained the patient had never been into any of the districts in which *G. palpalis* is known to occur. Exactly

* Castellani. 'Researches on the Etiology of Sleeping Sickness,' Jour. of Trop. Med. and Hyg., p. 167, 1903.

Thomas and Linton. 'A comparison of the animal reactions of the Trypanosomes of Uganda and Congo Free State Sleeping Sickness with those of *T. gambiense*,' Lancet, May 14, 1904.

Plimmer. 'Note on the effect produced on Rats by the trypanosomata of Gambian fever and Sleeping Sickness,' Proc. Roy. Soc., No. 504, p. 388, 1905.

Laveran. 'Sur trois virus de trypanosomiase humaine de provenances differentes,' Comptes Rendus de l'Acad. Sciences, p. 1065, 1906.

Gray and Tulloch. Reports of the S.S. Commission of the Royal Society, No. 8, p. 53.

Bentmann und Günther. 'Beiträge zur Kenntnis des *T. gambiense*,' Archiv für Schiffs- und Tropenhygiene, B. XI, Beiheft 2, 1907.

† Bevan and MacGregor. 'Note on the passage of a Human Trypanosome through domestic animals,' Journal of Comparative Pathology and Therapeutics, p. 160, 1910.

where the disease was contracted it is impossible to say; possibly, as the patient himself thought, it was in the Luangwa Valley.

Stephens and Fantham* have described certain morphological peculiarities, which the parasite exhibits in the blood of rats, rabbits and guinea-pigs infected with this strain. As these peculiarities have never been found in *T. gambiense*, the authors consider that this Rhodesian trypanosome is different from *T. gambiense*, and, in fact, a new species, for which they suggest the name *T. rhodesiense*.

In view of these morphological differences and of the fact that the disease was acquired in a district where *G. palpalis* has not yet been found, it appeared desirable to examine the pathogenicity of the parasite for animals of different species, with the object of comparing it with that of our laboratory strain of *T. gambiense* and with the results obtained by other workers with various strains of human trypanosomes.

Recent work has indicated that experiments upon the pathogenicity of a trypanosome must be conducted with certain precautions.

It was found by Thomas and Breinl† that a strain of *T. gambiense*, after passage through a baboon, had greatly increased in virulence. They state that rabbits, rats, cats, guinea-pigs and monkeys inoculated with the strain derived from the baboon developed the disease after short incubation periods, and that death occurred early.

Breinl and Nierenstein‡ found occasional variations in the virulence of *T. gambiense*. Rats infected from a monkey died in three to four days. After passage through guinea-pigs, however, the heightened virulence of the strain was lost and it killed rats again in the normal period, namely, forty to one hundred and twenty days.

Again, it is now generally recognised that long continued passage of a trypanosome through one species of animal tends to increase the virulence of the parasite for this species.

* 'On the peculiar morphology of a trypanosome from a case of Sleeping Sickness and the possibility of its being a new species (*T. rhodesiense*),' Proc. Roy. Soc., Vol. LXXXIII, p. 28, 1910.

† 'Trypanosomes, Trypanosomiasis and Sleeping Sickness,' Liverpool School of Tropical Medicine, Memoir XVI, 1905.

‡ 'Bio-Chemical and Therapeutical Studies on Trypanosomiasis,' Annals of Trop. Med. and Parasit., Vol. III, p. 395, 1909.

The interesting observation of Schilling and Jaffé*, that mice, infected with a strain of Ngana, which had been preserved by passage through mice and subsequently treated with arsenophenylglycine, were temporarily immune to the mouse passage strain, but became infected on being inoculated with the same strain which had been kept up in guinea-pigs for two years, shows in what way the parasite can be modified by long passage through distinct animal species.

The work of Uhlenhuth, Hübner and Woithet† indicates that the quantity of infective blood injected may play an important rôle in determining the character of the resulting infection.

These authors, working with *T. equiperdum*, found that after inoculation of large quantities of infective blood, infection was delayed, and sometimes did not occur. Similar observations were made by Beck‡ with *T. gambiense*, who explains the fact on the ground that anti-bodies present in the infective blood act as trypanocides.

The strain which I have used in the following experiments was obtained by inoculating rats with the blood of the patient shortly after his arrival in England, before any medicinal treatment had been administered. The direct strain has been preserved by passage from rat to rat.

Before experiments on animals or other species were commenced, the strain had been passed through a series of twenty rats during a period of about six months.

For our laboratory strain of *T. gambiense* we are indebted to Professor Mesnil, who has kindly informed me that it was obtained by inoculating rats with the cerebrospinal fluid of a case of human trypanosomiasis from the French Congo, in 1905. Since then it has been kept up by passages through rats. Professor Mesnil adds that the strain has gradually become more virulent for rats, which it now kills on an average in twelve days.

* 'Weitere chemotherapeutische Versuche bei Trypanosomenkrankheiten,' Archiv. für Schiffs- und Tropenhygiene, S. 525, 1909.

† 'Experimentelle Untersuchungen über Dourine mit besonderer Berücksichtigung der Atoxylbehandlung,' Arb. a. d. Kaiserl. Gesundheitsamte, B. XXVII, S. 256, 1908.

‡ 'Experimentelle Beiträge zur Infektion mit *Trypanosoma gambiense* und zur Heilung der menschlichen Trypanosomiasis,' Arb. a. d. Kaiserl. Gesundheitsamte, S. 313, 1910.

In my experiments, the different animals were always inoculated with the blood of infected rats, so as to avoid any alteration in virulence comparable to that described by Thomas and Breinl, resulting from passage through a baboon.

With a view to avoiding any discrepancy arising from injection of widely varying numbers of trypanosomes, the injected blood was diluted with citrated saline solution, until it contained a definite number of parasites per cubic millimetre.

This was accomplished by diluting the blood of a heavily infected rat with many times its volume of citrated salt solution and then counting the trypanosomes in the diluted solution by means of the Thoma-Zeiss haemocytometer. The mixture was then further diluted with normal saline until one cubic millimetre contained 500, 1,000, 5,000 or 10,000 trypanosomes.

When cover-slip preparations of such solutions containing 1,000 trypanosomes per c.mm. were examined microscopically, it was found that there was approximately one parasite to a field (Zeiss, objective DD; eyepiece No. 4).

MONKEYS.

Rhodesian strain. Six experiments. The following species were used, *Macacus rhesus*, *Cercopithecus callitrichus* and *Cercopithecus ruber*. In all, the disease ran a very rapid course. Trypanosomes appeared in the blood after short incubation periods, and were subsequently always present—often in very large numbers—until death occurred. Posterior nuclear forms* of the parasite were found in every case except Monkey 6 (*Cercopithecus ruber*), where the parasites, although constantly present, were never numerous, the largest number found being ten to a field. With the exception of emaciation the animals presented no symptoms until within two or three days of death, when they lay at the bottom of their cages in a drowsy condition, ate little, if anything, and hardly responded to stimulation. The temperature never rose above

* Forms in which the nucleus was either close to the blepharoplast or actually posterior to it.

104° F. in any of the animals, but usually fell considerably shortly before death. Incubation period, three to five days; average four days. Duration, eight to fourteen days; average eleven days.

TABLE 1.—Monkeys infected with the *Rhodesian Strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. <i>Macacus rhesus</i> . Weight 2210 g.	Intraperitoneally with 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	5th day	14th day	Parasites always present in large numbers. Many posterior nuclear forms found. Weight at time of death, 1850 g.
2. <i>M. rhesus</i> . Weight, 1670 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd "	11th "	Parasites numerous on the sixth and seventh days. Posterior nuclear forms present. Subsequently the number of trypanosomes diminished. Weight at time of death, 1500 g.
3. <i>M. rhesus</i> . Weight, 5370 g.	Subcutaneously. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	4th "	9th "	Parasites present in large numbers. Posterior nuclear forms found. Weight at time of death, 5000 g.
4. <i>Cercopithecus callitricbus</i> Weight, 2520 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	4th "	10th "	Parasites present in considerable numbers from the sixth day until death. A few posterior nuclear forms seen. Weight at time of death, 2100 g.
5. <i>C. callitricbus</i> . Weight, 3160 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	4th "	8th "	Parasites numerous. Many posterior nuclear forms found. Weight at time of death, 2550 g.
6. <i>C. ruber</i> . Weight, 4150 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	4th "	14th "	Parasites fairly numerous (10 to a field) on the ninth day; otherwise scanty. No posterior nuclear forms found. Weight at time of death, 3600 g.

T. gambiense. Four experiments. *Macacus rhesus* and *Cercopithecus callitrichus* were used. With this strain the disease ran a much more chronic course. Parasites were usually scanty, or absent from the peripheral circulation. Incubation period, three to twenty-one days; average twelve days. Duration, twenty-seven days to over four months.

TABLE 2.—Monkeys infected with *T. gambiense*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. <i>Macacus rhesus</i> . Weight, 2490 g.	Intraperitoneally with 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	3rd day	27th day	Parasites always scanty or absent. Weight at time of death, 2130 g.
2. <i>M. rhesus</i> . Weight, 3380 g.	" "	5th "	91st "	Parasites scanty or absent generally, but fairly numerous during the last few days. Weight at time of death, 2600 g.
3. <i>Cercopithecus callitrichus</i> Weight, 2450 g.	" "	21st "	149th "	Parasites as a rule scanty or absent. Animal very drowsy during last week. Weight at time of death, 1820 g.
4. <i>C. callitrichus</i> . Weight, 2730 g.	Intraperitoneally with 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	6th "	108th "	Parasites occasionally present in large numbers, but usually scanty or absent. Weight at time of death, 1750 g.

RABBITS.

Rhodesian strain. Eight experiments. In these animals the disease was fairly acute. Incubation period, from three to fourteen days; average eight days. Duration, nineteen to forty-five days; average twenty-eight days. Parasites were almost constantly present in the blood, although usually only in small numbers, except during the last few days, when they were generally present in considerable numbers. Posterior nuclear forms were usually to be found in small numbers on the occasions when the blood contained numerous parasites.

The symptoms presented were those usually found in rabbits suffering from trypanosomiasis, viz., blepharo-conjunctivitis, oedema of the head and ears, purulent discharge from the nose and eyes, loss of hair, emaciation and anaemia. Probably, owing to the shorter course of the disease, these symptoms were not so marked as in the rabbits infected with *T. gambiense*, where the disease is much more chronic. Two of the animals were very drowsy for a week before death (Nos. 5 and 6).

TABLE 3.—Rabbits infected with the *Rhodesian strain*

Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	9th day	45th day	Parasites scanty until the last few days, when they were fairly numerous, and when a few posterior nuclear forms were seen.
2. Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd "	32nd "	" "
3. Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	7th "	26th "	" "
4. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	5th "	26th "	" "
5. " "	9th "	19th "	Parasites scanty, but constantly present until time of death, when they were fairly numerous (10 to a field). No posterior nuclear forms found. The animal was very lethargic during the last week.
6. " "	9th "	19th "	" "
7. Intraperitoneally. 1 c.c. of diluted blood solution containing 250 trypanosomes per c.mm.	14th "	—	Animal alive after 32 days. Parasites constantly present, but scanty. No posterior nuclear forms seen. Marked oedematous swelling of skin of head, loss of hair, ears thickened.
8. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	8th "	30th "	Parasites only found in small numbers until last few days, when they were fairly plentiful (5 to a field). A few posterior nuclear forms found.

T. gambiense. Eight experiments. The disease was chronic. Parasites were only occasionally found in the peripheral blood, and always in very small numbers. Incubation, five to nineteen days; average twelve days. In one animal parasites were never found in the blood during the whole course of the disease. It presented the usual symptoms of infection, viz., oedema, loss of hair, muco-purulent discharge from nose and eyes, etc. Duration, sixty days to more than five months.

TABLE 4.—Rabbits infected with *T. gambiense*

Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Intraperitoneally. 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	5th day	125th day	Parasites only occasionally found, and always scanty.
2. Intraperitoneally. 2 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	—	60th "	Parasites never found in blood. It presented the usual signs of disease.
3. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	9th "	140th "	Parasites rarely found, and only in very small numbers.
4. " "	15th "	109th "	" "
5. Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	7th "	—	Animal alive after five months. It presents marked symptoms of disease, e.g., loss of hair, etc. Parasites rarely present in blood.
6. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	19th "	—	Animal alive after three months. Parasites occasionally found in small numbers.
7. " "	15th "	—	Animal alive after 64 days. Parasites rarely found, and very scanty.
8. " "	—	—	Parasites have not yet been found in blood. Animal perfectly normal after 23 days.

GUINEA-PIGS.

Rhodesian strain. Six experiments. Parasites generally present in the blood, often in considerable numbers. Posterior nuclear forms were usually found when the parasites were numerous. The animals presented no symptoms, and the disease ran a fairly chronic course. Incubation period, three to fifteen days; average eight days. Duration, thirty-nine to eighty-two days; average fifty-five.

TABLE 5.—Guinea-pigs infected with the *Rhodesian strain*

Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	15th day	41st day	Parasites scanty except during the last week, when they were fairly numerous (15 to 20 to a field). Posterior nuclear forms found.
2. Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd "	52nd "	" "
3. " "	12th "	39th "	Parasites present in blood regularly, but in small numbers. No posterior nuclear forms seen.
4. " "	7th "	39th "	Parasites constantly present, generally in large numbers. Many posterior nuclear forms found.
5. Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	6th "	82nd "	" "
6. Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd "	76th "	Parasites always scanty until the last few days, when they were fairly numerous (10 to a field). Posterior nuclear forms seen.

T. gambiense. Five experiments. Parasites usually present only in very small numbers or absent. Disease very chronic. Incubation period, three to forty days; average fifteen days. Duration, forty-two days to five and a half months; average more than four months.

TABLE 6.—Guinea-pigs infected with *T. gambiense*

Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Intraperitoneally. 1 c.c. of diluted blood solution containing (?) trypanosomes per c.mm.	40th day	134th day	Parasites not found in blood (except on 40th day) until the last two days, when they were present in small numbers.
2. " "	3rd "	167th "	Parasites rarely found in small numbers.
3. Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	7th "	42nd "	Parasites regularly present, and sometimes in considerable numbers (20 to 30 to a field).
4. " "	13th "	—	Parasites only found on three occasions in very small numbers. Animal alive and well after four months.
5. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	12th "	160th "	Parasites rarely found in very small numbers.

DOGS.

Rhodesian strain. Four experiments. The trypanosome proved to be extremely virulent in dogs. In Dogs 1 and 2, parasites were present in the blood in large numbers, and many posterior nuclear forms were seen. Trypanosomes, although constantly present, were never very numerous in Dogs 3 and 4, and none of the above varieties were observed. The animals rapidly lost their weight. Incubation, four to five days; average four days. Duration, nine to thirteen days; average twelve days.

TABLE 7.—Dogs infected with the *Rhodesian strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Mongrel. Weight, 5100 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	4th day	9th day	Parasites present in large numbers. Posterior nuclear varieties seen. Weight at time of death, 4300 g.
2. Irish terrier. Weight, 12,050 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 250 trypanosomes per c.mm.	5th "	13th "	Parasites present in large numbers. Posterior nuclear forms seen. Weight at time of death, 10,460 g.
3. Collie Weight, 7950 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	4th "	13th "	Parasites 10 to a field on the sixth to ninth days. Subsequently they were scanty. No posterior nuclear forms found. Weight at time of death, 7150 g.
4. Spaniel. Weight, 17,090 g.	" "	4th "	12th "	Parasites scanty. No posterior nuclear forms seen. Weight at time of death, 15,020 g.

T. gambiense. Two experiments. In both animals the disease ran a chronic course. Parasites were, as a rule, only found in small numbers. Incubation period, twelve to sixteen days; average fourteen.

TABLE 8.—Dogs infected with *T. gambiense*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Irish terrier. Weight, 9500 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	12th day	64th day	Parasites found fairly regularly in small numbers. On the last two days trypanosomes were present in large numbers (30 to a field).
2. Mongrel. Weight, 10,250 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	16th "	—	Parasites found fairly constantly, but usually in very small numbers. Animal still alive on 34th day.

GOATS.

Rhodesian strain. Three experiments. In these animals the disease ran a fairly rapid course. Parasites were not infrequently seen in the blood, but in very small numbers only, and the posterior nuclear variety was never found. The only symptoms observed were: loss of weight, oedematous swellings—accompanied by slight loss of hair—of the skin and subcutaneous tissues over the nasal bones and beneath the eyes, and interstitial keratitis. The temperature of Goat 1 rose to 107° F. on the seventh day after inoculation, but quickly returned to normal (103.4° F.). The other two animals never exhibited any rise of temperature. Incubation period, four to eleven days; average seven days. Duration, forty-five to fifty-five days; average eight days.

TABLE 9.—Goats infected with the *Rhodesian strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Medium ♀	Subcutaneously. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	6th day	55th day	Parasites scanty. No posterior nuclear forms seen.
2. Large ♀	Intraperitoneally. 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	4th "	46th "	Parasites scanty. No posterior nuclear forms seen.
3. Large ♂	Subcutaneously. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	11th "	45th "	Parasites scanty and rarely found. No posterior nuclear forms seen.

T. gambiense. Two experiments. Both animals became infected, but the disease was exceedingly chronic. Parasites have never been found in the blood of Goat 1, but two rats, inoculated with its blood on the forty-fourth day, became infected after an incubation period of twelve days. The animal is still alive, more than five months after inoculation, and has presented no symptoms of disease. Rats inoculated with its blood, on the hundred and

fifty-second day, did not become infected. Parasites (1 to 15 fields) were found in the blood of Goat 2 on the nineteenth day after inoculation, but were never observed subsequently. On the seventy-second day slight swelling of the face was noticed. Beyond slight wasting no other symptom was exhibited. Death occurred on the eighty-fifth day.

TABLE 12.—Goats infected with *T. gambiense*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Large ♀	Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	—	—	Parasites never found in blood. Rats inoculated with its blood on the 44th day became infected, but not those inoculated on the 152nd day. Animal alive and well after more than five months.
2. Medium ♀	Intraperitoneally. 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	19th day	85th day	Parasites only found once (on the 19th day) in very small numbers. Rats inoculated with its blood on the day of death became infected.

DONKEY AND HORSE.

Rhodesian strain. Two donkeys and a horse were easily infected.

Donkey 1. Inoculated subcutaneously with a small amount of the virus. Parasites first appeared in the circulation on the fifth day. From the sixth to the eighth day trypanosomes were present in fair numbers (3 to 10 to a field), and a few posterior nuclear forms were found. Subsequently parasites were constantly present in very small numbers until the last week when the number again increased, and the posterior nuclear variety were again found. Temperature remained constant (103.4° F.) throughout the disease. Beyond wasting and an oedematous condition of the skin and

subcutaneous tissues of the face, there were no symptoms, until the forty-seventh day, when it was noticed that the animal was very weak. The next day it was down and unable to rise. Death occurred on the fifty-fourth day.

TABLE 11.—Donkeys and Horse infected with the *Rhodesian strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Donkey ♀ Weight, 364 lbs.	Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	5th day	54th day	Parasites constantly present, but, as a rule, scanty, except on last three days, when they were fairly numerous (10 to 20 to a field). Posterior nuclear forms found. Weight a week before death, 290 lbs.
2. Donkey ♂ Weight, 324 lbs.	Subcutaneously. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	7th "	28th "	Parasites constantly present in small numbers. No posterior nuclear forms found. Weight five days before death, 280 lbs.
3. Pony ♂ Weight, 644 lbs.	Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	7th "	38th "	Parasites constantly present, but only in small numbers until within a few days of death, when 3 to 5 to a field were found. A few posterior nuclear forms were seen. Weight three days before death, 548 lbs.

Donkey 2. Inoculated subcutaneously. Incubation period seven days. Parasites were subsequently constantly present in the blood in small numbers. No posterior nuclear forms were seen. Temperature 103-104° F. The only signs of disease were progressive wasting and debility. Death took place on the twenty-eighth day.

A *Horse* inoculated subcutaneously with a small dose of the Rhodesian strain exhibited parasites in its blood on the seventh day. Trypanosomes were subsequently regularly found, there

being sometimes as many as five to a field, but, as a rule, they were scanty. A few posterior nuclear forms were found on the thirty-second day, at a time when the total number of parasites in the blood was about five to a field. The animal rapidly lost weight. Before infection it weighed 650 lbs., five days before death 548 lbs. The temperature remained constant, between 103° and 103.8° F., until a few hours before death, when it fell to 99° F. On the twenty-fourth day after inoculation the animal appeared ill for the first time. A few days later distinct swelling of the skin, subcutaneous tissues below the eyes and over the nasal bones was noticed. This condition was accompanied by some slight loss of hair and persisted until death. On the thirty-fifth day the animal was very ill. It had lost nearly 100 lbs. in weight, could hardly stand and refused food. The cornea of the left eye was hazy, and the conjunctiva slightly injected. The next day the animal was down and could not get up. Death occurred on the thirty-eighth day. At the time of death parasites were present in the blood in fair numbers (4-5 to a field).

MICE.

Rhodesian strain. Twenty experiments. The disease ran an acute course. The animals were inoculated intraperitoneally with $\frac{1}{4}$ c.c. of blood containing approximately 500 trypanosomes to a cubic millimetre. Parasites were usually present in fair numbers, and the posterior nuclear forms frequently found. Incubation period, two to five days; average three days. Duration, three to twenty days; average eleven days.

RATS.

Rhodesian strain. Forty-five experiments. The strain has been preserved by passage through these animals. In each case the rats were inoculated intraperitoneally with 1 c.c. of infective blood well diluted with citrated saline solution—a cover-slip preparation of which showed, as a rule, one to five trypanosomes to a field. The disease was fairly acute, and parasites were often present in the blood in considerable numbers. Posterior nuclear forms were usually found when the blood contained numerous parasites. Very

little alteration in virulence has been observed after a year's passage through these animals. The two rats infected directly from the patient died in six and twelve days, respectively. The average duration of the disease in the first ten passages was 17.5 days, and in the last ten, 20 days. Incubation period, one to seven days; average four days. Duration, six to forty-five days; average nineteen days.

T. gambiense. Fifty-four experiments. Incubation period, two to ten days; average four days. Duration, six to twenty-nine days; average fourteen days.

With a view to comparing the animal reactions of the *Rhodesian Trypanosome* with those of *T. gambiense*, the above results, and those obtained by other workers* with various strains of human trypanosomes, are summarised in Table 12.

It is evident that the Rhodesian trypanosome differs very markedly from *T. gambiense* in its pathogenicity for various species of animals. In monkeys (*Macacus rhesus*, *Cercopithecus callitrichus* and *Cercopithecus ruber*), dogs, rats and mice the disease runs a very acute course. Large numbers of trypanosomes were usually present in the blood. Rabbits, guinea-pigs and goats were easily infected, and died in a much shorter time than animals of the same species infected with *T. gambiense*, and parasites were much more constantly found in the blood and in greater numbers. Two donkeys and a horse quickly succumbed to the disease, trypanosomes being regularly observed in the peripheral circulation.

These results show that in so far as virulence is concerned the parasite is widely different from *T. gambiense*—in fact it more closely resembles *T. brucei* in this respect than *T. gambiense*—and support the view advanced by Stephens and Fantham, on morphological grounds, that we have in '*T. rhodesiense*' a new species.

* Reports of the Sleeping Sickness Commission of the Royal Society.

Thomas and Linton. *Loc. cit.*

Laveran. *Loc. cit.*

Brumpt et Wurtz. 'Maladie du Sommeil expérimentale chez les Souris, Rats, Cobayes, &c.' *Comptes Rendus Soc. Biologie*, T. LVI, p. 567, 1904.

Beck. 'Experimentelle Beiträge zur Infektion mit *Trypanosoma gambiense*,' *Arbeiten a. d. Kaiserl. Gesundheitsamte*, S. 318, 1910.

Bentmann und Günther. *Loc. cit.*

TABLE 12. Summary of the results obtained in various animals with the *Rhodesian strain* and *T. gambiense* respectively, and those of other workers with various strains of *T. gambiense*

	<i>Rhodesian strain</i>		<i>T. gambiense</i>		RESULTS OBTAINED BY VARIOUS WORKERS WITH DIFFERENT STRAINS OF HUMAN TRYPANOSOMES											
	Incubation	Duration	Incubation	Duration	Royal Society Commissioners in Uganda		Thomas and Linton		Laveran		Brumpt and Wurtz		Beck		Bentmann and Günther	
					Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration
Rat	4 days	19 days	4 days	14 days	20 days	3-8 +mths.	8 days	45-388 days	G.* 13 days U. 11 " K. —	62 days 77 " 81 "	3-4 days	4-5 months	3-5 days	3-4 mths.	U. 4.9 days K. 12.5 "	69 days 81 "
Mouse	3 "	11 "	—	—	—	—	4-7 days	11-14 days	G. 13 " U. 8-20 " K. 30-45 "	57 " 153-216 " 281-351 "	2-3 "	3-4 "	2-3 "	8-10 days	U. 5.3 " K. 4.9 "	49 " 90 "
Guinea-pig	8 "	55 "	15 "	3-5 +mths.	—	—	12-45 "	3-8 mths.	G. 26 " U. 16 " K. 20 "	100 " 81 " 100 "	Always negative	1½ "	2-3 "	3-5 mths.	U. six experiments with no positive result K. only 2 positive results out of 21 experiments	
Rabbit	8 "	28 "	12 "	3-5 +mths.	—	—	5-15 "	54-128 days	—	—	Always negative	1-4 + "	8-14 "	4-5 mths.	U. 9 " K. 9 "	28.6 days 49 "
Dog	4 "	12 "	14 "	More than 1½ months	22 "	Some months	5-10 "	33-43 "	—	—	17 days	66 days	2-3 "	1½-4 mths.	—	—
Monkey— <i>Macacus rhesus</i>	4 "	11 "	4 "	1-3 mths.	9-22 days	2-8 mths.	—	—	—	—	8-9 "	16 days to 2 months	—	—	—	—
<i>Cercopithecus callitricibus</i>	4 "	9 "	13 "	3-5 "	9-40 "	2½-12 "	—	—	—	—	7 "	19-88 + days	—	—	U. 10 " K. 12 "	82 " 105 "
<i>Cercopithecus ruber</i>	4 "	14 "	—	—	—	—	—	—	—	—	7 "	24 days	—	—	—	—
Goat	7 "	48 "	Parasites only found once on 19th day in Goat 1; never found in Goat 2	3-5 +mths.	Parasites found on 13th day after 5th inoculation in one goat. Other 2 goats negative	One animal died on 22nd day. Other two recovered	—	All died: duration not stated	—	—	—	—	—	—	—	—
Donkey	6 "	41 "	—	—	3 were inoculated, but did not become infected	—	Parasites found on rare occasions	—	—	—	—	—	—	—	—	—
Horse	7 "	38 "	—	—	—	—	30 days	Recovered	—	—	—	—	—	—	—	—

* G. signifies *T. gambiense*, from Gambia; U. Trypanosome of Sleeping Sickness in Uganda; K. Trypanosome of Sleeping Sickness in Congo.

My observations as to the morphology of the parasite confirm those of Stephens and Fantham regarding the existence of posterior nuclear forms in infected rats, guinea-pigs and rabbits. I have also found them in monkeys (*Macacus rhesus*, *Cercopithecus callitrichus*), dogs, mice, horse and donkey.

I have not, however, succeeded in finding these forms in the blood of the patient himself, in spite of very careful daily examinations during a period of over three months.

Why the posterior nuclear forms should be apparently absent from the patient's blood is not very obvious. Trypanosomes were always scanty and possibly these forms may have been missed, although nearly two thousand parasites were examined without meeting a single example of the form in question. However, the posterior nuclear variety is not usually seen in infected animals until the blood is fairly heavily infected, when they may form as many as five or six per cent. of the total parasites present.

Sections of the nervous system of the patient exhibit well-marked perivascular infiltration and leptomeningitis. The spleen was greatly increased in size and deeply pigmented (malarial?). The cervical, axillary, mesenteric and inguinal lymphatic glands were considerably enlarged, and presented the usual histological appearances.